Chromatography

By-

Dr. Ekta Khare

Chromatography

- The word chromatography means "color writing" which is a way that a chemist can test liquid mixtures.
- While studying the coloring materials in plant life, a Russian botanist invented chromatography in 1903.
- His name was M.S. Tswett.

PRINCIPLES OF CHROMATOGRAPHY

Distribution coefficients

- The basis of all forms of chromatography is the distribution or partition coefficient (Kd), which describes the way in which a compound (the analyte) distributes between two immiscible phases.
- For two such phases A and B, the value for this coefficient is a constant at a given temperature and is given by the expression:

$$\frac{\text{concentration in phase A}}{\text{concentration in phase B}} = K_d$$

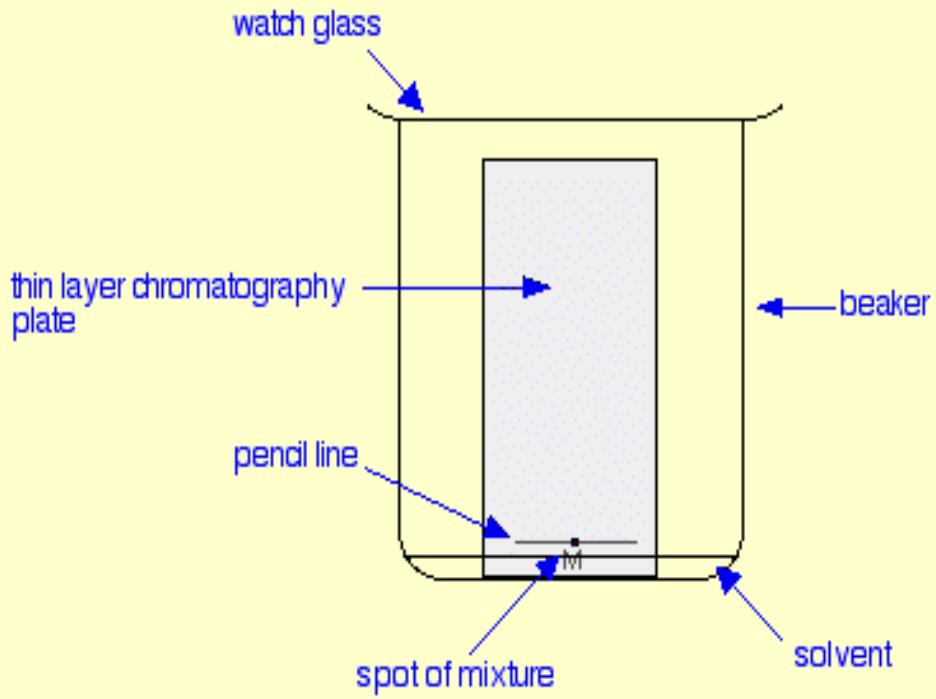
Effective Distribution Coefficient

- The term effective distribution coefficient is defined as the total amount, as distinct from the concentration, of analyte present in one phase divided by the total amount present in the other phase.
- It is in fact the distribution coefficient multiplied by the ratio of the volumes of the two phases present.
- If the distribution coefficient of an analyte between two phases A and B is 1, and if this analyte is distributed between 10 cm^3 of A and 1 cm^3 of B, the concentration in the two phases will be the same, but the total amount of the analyte in phase A will be 10 times the amount in phase B.

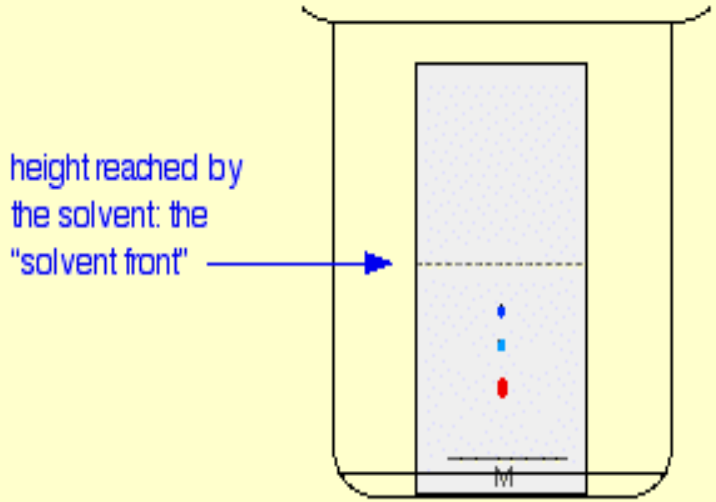
- All chromatographic systems consist of the:
  - stationary phase, which may be a solid, gel, liquid or a solid/liquid mixture that is immobilised, and
  - the mobile phase, which may be liquid or gaseous, and which is passed over or through the stationary phase after the mixture of analytes to be separated has been applied to the stationary phase.
- During the chromatographic separation the analytes continuously pass back and forth between the two phases so that differences in their distribution coefficients result in their separation.

Thin layer Chromatography

- Thin layer chromatography is performed on a sheet of glass, plastic, or aluminum foil, which is coated with a thin layer of adsorbent material, usually silica gel, aluminum oxide, or cellulose (blotter paper).
- This layer of adsorbent is known as the stationary phase.
- After the sample has been applied on the plate, a solvent or solvent mixture (known as the mobile phase) is drawn up the plate via capillary action.
- Because different analytes ascend the TLC plate at different rates, separation is achieved.
- The principle of TLC is the distribution of a compound between a solid fixed phase (the thin layer) applied to a glass or plastic plate and a liquid mobile phase (eluting solvent) that is moving over the solid phase.
- A small amount of a compound or mixture is applied to a starting point just above the bottom of TLC plate.



Note: The chromatography plate will in fact be pure white - not pale grey. I'm forced to show it as off-white because of the way I construct the diagrams. Anything I draw as pure white allows the background colour of the page to show through.



The diagram shows the plate after the solvent has moved about half way up it.

TLC

- It may be performed on the analytical scale as a means of monitoring the progress of a reaction, or on the preparative scale to purify small amounts of a compound.
- TLC is an analytical tool widely used because of its simplicity, relative low cost, high sensitivity, and speed of separation.
- TLC functions on the same principle as all chromatography: a compound will have different affinities for the mobile and stationary phases, and this affects the speed at which it migrates.
- The goal of TLC is to obtain well defined, well separated spots.
- The mobile phase has different properties from the stationary phase. For example, with silica gel, a very polar substance, non-polar mobile phases such as heptane are used.
- The mobile phase may be a mixture, allowing chemists to fine-tune the bulk properties of the mobile phase.
- After the experiment, the spots are visualized. Often this can be done simply by projecting ultraviolet light onto the sheet; the sheets are often treated with a phosphor, and dark spots appear on the sheet where compounds absorb the light impinging on a certain area.
- Chemical processes can also be used to visualize spots; anisaldehyde, for example, forms colored adducts with many compounds, and sulfuric acid will char most organic compounds, leaving a dark spot on the sheet.

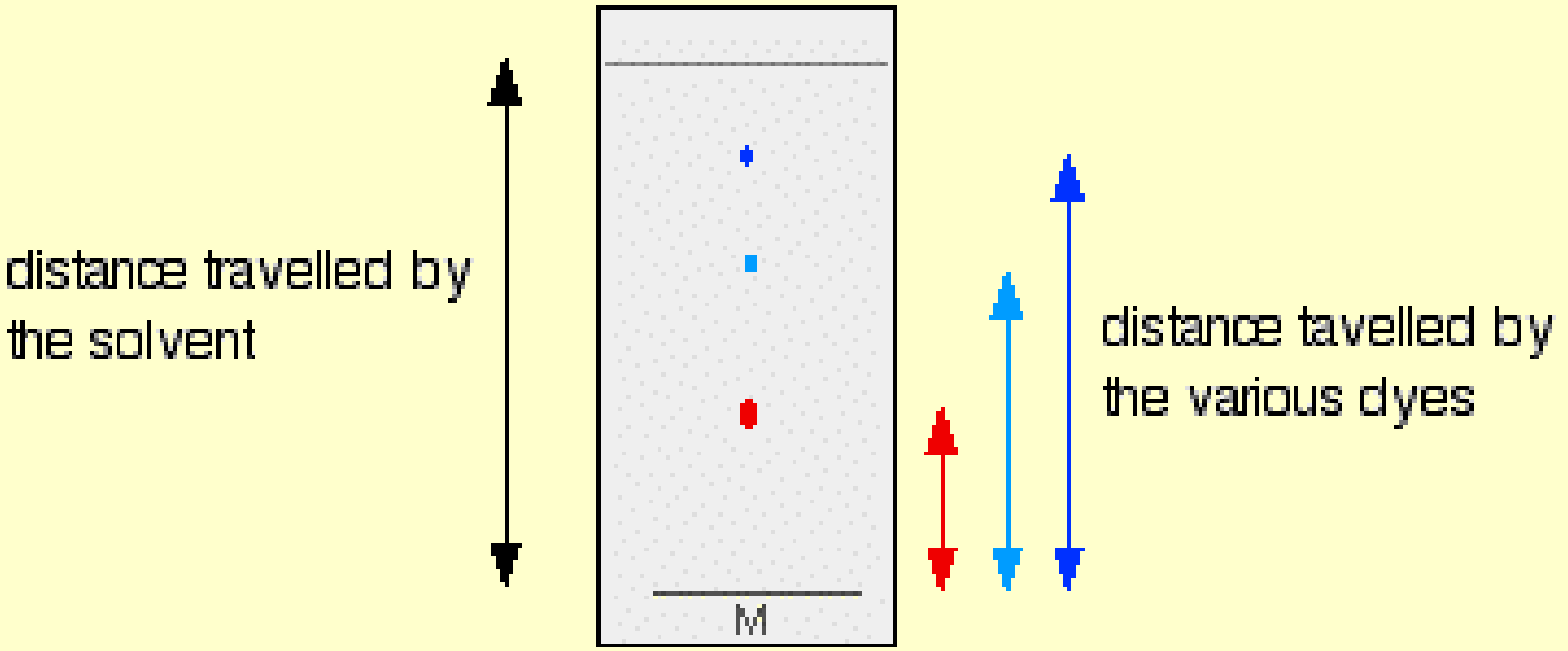
TLC

- Whether the compound moves up the plate or stays behind depend on the physical properties of that individual compound and thus depend on its molecular structure, especially functional groups.
- The solubility rule **“ Like Dissolves Like”** is followed.
- The more similar the physical properties of the compound to the mobile phase, the longer it will stay in the mobile phase.
- The mobile phase will carry the most soluble compounds the furthest up the TLC plate.
- The compounds that are less soluble in the mobile phase and have a higher affinity to the particles on the TLC plate will stay behind.

Retardation factor (Rf) values

- The behavior of an individual compound in TLC is characterized by a quantity Known as Rf and is expressed as a decimal fraction.
- The Rf is calculated by dividing the distance the compound travelled from the original position by the distance the solvent travelled from the original position (the solvent front).

$$\mathbf{Rf} = \frac{\text{Distance of centre of spot from starting point}}{\text{Distance of solvent front from starting point}}$$


distance travelled by
the solvent
M
distance tavelled by
the various dyes

The Rf value is a constant for each component only under identical experimental condition. It depends upon number of factors as:

- **Nature of adsorbent**: Different adsorbents will give different Rf value for same solvent. Reproducibility is only possible for given adsorbent of constant particle size and binder. Plates should be stored over silica gel in desiccators before use and the sample should be applied quickly so that the water vapor in the atmosphere is not adsorbed by the plate. Because of the difficulties associated with activation procedures, it is far better to use plates stored at room temperature and not to activate them.
- **The mobile phase**: The purity of solvents and quantity of solvent mixed should be strictly controlled. It should be made freshly for each run if one of the solvents is very volatile or hygroscopic. Example- acetone.
- **Temperature**: Although precise control of temperature is not necessary, the tank should be kept away from sources of heat, direct sunlight etc. As the temperature is increased, Volatile solvents evaporate more quickly, solvents run faster, and Rf values generally decrease slightly.
- **Thickness of layer**: Standard plates approximately 250 micrometer is the preferable thickness of layer. Below 200, the Rf values vary considerably. The layers may be of higher or lower thickness in individual compounds.

Factors affecting Rf value

- **Developing tank**: It is important that saturated conditions are attained for running TLC plates. This is best accomplished by using small tanks with filter paper liners and sufficient solvent, and by leaving the tank to equilibrate for at least 30 minutes before running the plates. A well fitting lid is essential.
- **Mass of sample**: Increasing the mass of sample on the plate will often increase the Rf of drug, especially if it normally tails in the system. However, if a plate is grossly overloaded, this too will give a tailing spot and will have the effect of apparently decreasing the Rf value. The two situations are normally easy to distinguish by the intensity of the spot.

Applications

- Thin layer chromatography has been a useful tool in numerous applications of pharmaceutical importance.
- **TLC of amino acids:** TLC of amino acids is more difficult than TLC of inks, because amino acids are colorless. Therefore, one cannot see the spots with the naked eye once the plate is fully developed and dried. To see the spots, it is necessary to use either the ninhydrin or the black-light visualization techniques.
- **Pharmaceuticals and drugs**: TLC is used in the identification, purity testing and determination of the concentration of active ingredients, auxiliary substances and preservatives in drugs and drug preparations, process control in synthetic manufacturing processes. Various pharmacopoeias have accepted TLC technique for the detection of impurity in a drug or chemical
- **Separation of multicomponent pharmaceutical formulations**: It is also used in separation of multicomponent pharmaceutical formulations.

...Applications

- **Qualitative analysis of alkaloids**: It is used in qualitative analysis of alkaloids in control phase of both pharmaceutical formulations and vegetable drugs. The spots are visualized by spraying first with an alcoholic iodine-potassium iodine solution followed by 25% HCl- 96% ethanol (1:1).
- **Clinical chemistry and Biochemistry**: For the determination of active substances and their metabolites in biological matrices, diagnosis of metabolic disorders such as phenylketonuria, cystinuria and maple syrup disease in babies. It serves as an useful tool in analysis of urinary constituent derived from lipids in analysis of many urinary constituents such as steroids, amino acids, porphyrins and bile acids.
- **Cosmetology:** In the identification of dye raw materials and end products, preservatives, surfactants, fatty acids, constituents of perfumes.
- **Food Analysis**: For the determination of pesticides and fungicides in drinking water, residues in vegetables, salads and meat, vitamins in soft drinks, banned additives in Germany (e.g. sandalwood extract in fish and meat products), compliance with limit values (e.g. polycyclic compounds in drinking water, aflatoxins in milk and milk products).

...Applications

- **Analysis of Heavy Petroleum Product**[8]: Thin-layer chromatography (TLC), which is commonly used in the analysis of complex mixtures, is seldom used in the investigation of petroleum products, maybe the most complex objects. TLC technique used (in the preparataive variant) for a rapid determination of the group composition of heavy petroleum products (asphalts, pitches, resids.
- **Applications related to Organic Chemistry**
- TLC has also been applied successfully in various purification processes, checking of distillation fractions and for checking the progress of purification by molecular distillation.